Technical Variations in Low-Input RNA-seq Methodologies.

Journal:	Sci Rep
Publication Year:	2014
Authors:	Vipul Bhargava, Steven R Head, Phillip Ordoukhanian, Mark Mercola, Shankar Subramaniam
PubMed link:	24419370
Funding Grants:	Interdisciplinary Stem Cell Training Program at UCSD II, Interdisciplinary Stem Cell Training Program at UCSD

Public Summary:

Recent advances in RNA-seq methodologies from limiting amounts of mRNA have facilitated the characterization of rare cell-types in various biological systems. So far, however, technical variations in these methods have not been adequately characterized, vis-à-vis sensitivity, starting with reduced levels of mRNA. Here, we generated sequencing libraries from limiting amounts of mRNA using three amplification-based methods, viz. Smart-seq, DP-seq and CEL-seq, and demonstrated significant technical variations in these libraries. Reduction in mRNA levels led to inefficient amplification of the majority of low to moderately expressed transcripts. Furthermore, noise in primer hybridization and/or enzyme incorporation was magnified during the amplification step resulting in significant distortions in fold changes of the transcripts. Consequently, the majority of the differentially expressed transcripts identified were either high-expressed and/or exhibited high fold changes. High technical variations ultimately masked subtle biological differences mandating the development of improved amplification-based strategies for quantitative transcriptomics from limiting amounts of mRNA.

Scientific Abstract:

Recent advances in RNA-seq methodologies from limiting amounts of mRNA have facilitated the characterization of rare cell-types in various biological systems. So far, however, technical variations in these methods have not been adequately characterized, vis-a-vis sensitivity, starting with reduced levels of mRNA. Here, we generated sequencing libraries from limiting amounts of mRNA using three amplification-based methods, viz. Smart-seq, DP-seq and CEL-seq, and demonstrated significant technical variations in these libraries. Reduction in mRNA levels led to inefficient amplification of the majority of low to moderately expressed transcripts. Furthermore, noise in primer hybridization and/or enzyme incorporation was magnified during the amplification step resulting in significant distortions in fold changes of the transcripts. Consequently, the majority of the differentially expressed transcripts identified were either high-expressed and/or exhibited high fold changes. High technical variations ultimately masked subtle biological differences mandating the development of improved amplification-based strategies for quantitative transcriptomics from limiting amounts of mRNA.

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